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Soil disinfestation with cruciferous amendments and sublethal heating: effects on *Meloidogyne incognita*, *Sclerotium rolfsii* and *Pythium ultimum*

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Controlled environment experiments were carried out to test the effects of amending soil with fresh and dried residues of certain cultivated and noncultivated cruciferous plants, including *Brassica nigra, B. oleracea* var. *chinensis, B. oleracea* var. *italiensis, B. oleracea* var. *capitata, B. oleracea* var. *compacta* and *Raphanus sativus*; and of a sublethal soil heating regime (38°C day/27°C night) on survival and activity of nematode and fungal plant pathogens including *Meloidogyne incognita*, *Sclerotium rolfsii* and *Pythium ultimum*. Addition of the various cruciferous amendments to soil without heating resulted in significantly reduced tomato root galling (38–100%) by *M. incognita* or reduced recovery of active fungal pathogens (0–100%) after 7 days incubation. When cruciferous soil amendments were combined with the sublethal heating regime, nematode galling was reduced by 95–100%, and recovery of active fungi was reduced by 85–100%. No differences were found between fresh or dried cruciferous residues.

Introduction

Soil solarization is used as a method of soil disinfestation on a relatively small but expanding scale worldwide for greenhouse, field, garden and plant growth media applications (Katan, 1987; Stapleton & DeVay, 1995; Stapleton, 1997). Solarization is a passive but complex mode of action comprised of physical, chemical and biological components. It has benefits and limitations, but is an effective soil disinfestant in warm geographic areas for both agricultural and horticultural applications. Several studies have shown that solarization may be productively combined with other chemical and biological control methods (Katan, 1987; Stapleton *et al.*, 1991; Gamliel & Stapleton, 1993a; Chellemi *et al.*, 1994; Stapleton & DeVay, 1995; Tjamos & Fravel, 1995).

One promising combination of organic amendments with solarization uses residues of cruciferous plants, which contain glucosinolates (Duncan, 1991), and can release a number of biotoxic volatile compounds into soil during the decomposition process. Typically, members of the Cruciferae have shown varying degrees of biocidal activity as green manures without combination with solarization (Lewis & Papavizas, 1971, 1974; Muehlchen *et al.*, 1990; Angus *et al.*, 1994; Mayton *et al.*, 1996; Lodha *et al.*, 1997). However, the level of activity is often relatively weak and unpredictable. When cabbage crop residues were combined with sublethal or lethal levels of heating from solarization, the deleterious effect on soilborne fungal phytopathogens including *Fusarium oxysporum* f.sp. *conglutinans, Pythium ultimum*, and *Sclerotium rolfsii* was much greater and much more consistent (Ramirez-Villapudua & Munnecke, 1987, 1988; Gamliel & Stapleton, 1993b) or subsequent crop growth was increased (Keinath, 1996). Controlled environment studies were conducted to compare the effects of soil amendment with preparations of several cultivated and wild species, alone and in combination with a sublethal heating regime, on the nematode and fungal plant pathogens *Meloidogyne incognita, P. ultimum* and *S. rolfsii.*

Materials and methods

Apparatus and inoculum preparation

Controlled-environment experiments were conducted in covered containers as previously described (Gamliel & Stapleton, 1993b) to test the effects of soil amendment with several preparations of cruciferous plant residues, alone or in combination with sublethal heating, on soilborne survival and activity of the plant pathogens *M. incognita*, *S. rolfsii*, and *P. ultimum*. The soil used was Hanford fine sandy loam (46% sand, 45% silt, 9% clay; pH 7·4) naturally infested with *M. incognita* (approximately 150 second-stage juveniles (J2) per L of soil) and *P. ultimum* (approximately 29 propagules

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(oospores) per g of soil). Laboratory-grown sclerotia of *S. rolfsii*, originally isolated from roots of diseased apple trees (*Malus domestica* 'Granny Smith') in Stanislaus County, CA, were added to the soils in mesh bags (30 sclerotia per bag) prior to treatment. Soil for treatment was loaded into wide-mouthed, 2 L capacity glass jars sealed with metal lids. Four subsamples of soil per test were heat-treated by incubation in a single modified, Wisconsin-type water bath with diurnal temperature maximum and minimum of 38°C and 27°C, respectively, while the nonheated subsamples were maintained in a similar water bath set at 23°C.

Cruciferous amendments

The cruciferous organic amendments used in these tests were: black mustard (*Brassica nigra*), bok choy (*B. oleracea* var. *chinensis*), broccoli (*B. oleracea* var. *italiensis*), cabbage (*B. oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *compacta*) and wild radish (*Raphanus sativus*). All amendments were incorporated into soil at a concentration of 2% (w/w), the approximate quantity of residues incorporated into soil at the end of a cropping cycle in commercial field production.

Determination of treatment effects

Effects of treatments on *M. incognita* were estimated after 7 days using a bioassay procedure: treated soil was aired in open plastic bags for 24 h following the incubation period, then placed in two 10 cm diameter pots per replication. A single plant of a susceptible tomato cultivar (*Lycopersicon esculentum* 'Cherry Belle') was transplanted into each pot and the pots were maintained in a glasshouse at 30°C maximum and 21°C minimum. After 6 weeks of growth, roots were washed and an arbitrary gall rating was made (0–4 scale, where 0 = no galls evident and 4 = 75–100% of roots galled by visual examination). Sensitivity of *S. rolfsii* to treatments was determined after incubation for 7 days, as previously described (Gamliel & Stapleton, 1993b), by retrieving and surface-disinfesting the 30 sclerotia from the experimental containers, then placing them on PDA agar plates to determine germinability (Gamliel & Stapleton, 1993b). After treatment periods of 0, 2, 4 and 7 days, effects on *P. ultimum* were determined by sampling soil from containers, then air-drying and plating it on a selective medium (Gamliel & Stapleton, 1993b). At least two sets of assays were performed for each amendment and soil heating combination tested.

Data analysis

In vitro experiments were used as replications for comparing amendments and sublethal heat treatment. For each experiment, subsamples were averaged for each amendment by combining results from the heat/no-heat treatments. All amendments were not included in each experiment, but each experiment had a non-amended control treatment. Consequently, the number of replicates differed among treatments. Data for pathogen survival and activity taken as percentages of the control treatments were transformed, using the arcsin of the square root or the base 10 logarithm to homogenize the variances. Analysis of variance was run for the controls to compare experiments and also to test the effects of amendments, heat treatments and their interactions. Contrasts of interest were calculated to test amendment versus control, dry versus fresh amendment where applicable, the interaction of amendment versus control and heat, and the interaction of the dry versus fresh comparison and heat. Fisher's Protected LSD test was used for mean separation of amendments for nonheated and also for heated treatments.

Results

Analysis of variance for controls showed that experiments were not significantly different for galling of tomato roots by *M. incognita* or for germination of *S. rolfsii* sclerotia after 7 days. Experiments were significantly different for *P. ultimum* survival; therefore survival of *P. ultimum* after exposure to the various amendments for 7 days was calculated relative to the corresponding non-amended control without heating for the respective experiment. Post-treatment population assays of each of the test organisms showed that the heating regime used during the experiments was sublethal (Tables 1, 2, 3 and Fig. 1). For each of the test pathogens, significant main effects for amendments, soil heating, and the amendment × heating interaction were found, with the exception that the interaction was not significant for *S. rolfsii*. The other consistently significant statistic across the range of pathogens was the contrast of amendment versus the control × heat, indicating that when amendments were incorporated into soil, they had more biotoxic activity when heated. Since the amendment × heat treatment interactions and/or the amendment versus control × heat contrasts were significant, LSD tests were used for mean separation of amendments for heated and also for nonheated treatments.

Addition of each of the cruciferous soil amendments alone gave significant reductions in root galling of tomato caused by *M. incognita* ranging from 38 to 100%, compared with the nonheated control, after treatment for 7 days. The most effective amendments when incorporated without heat were fresh and dried bok choy. Gall ratings were decreased by 95–100% when any of the amendments were combined with sublethal heating, with a significant treatment interaction between amendments and heating (Table 1). Addition of cruciferous amendments without heating reduced germination of *S. rolfsii* sclerotia by 0–65% after incubation for 7 days (Table 2). Soil amendment with bok choy, broccoli and cabbage caused the most

Table 1 Effect of soil amendment with fresh or dried cruciferous plant residues, and/or sublethal heating, on galling of tomato roots by *Meloidogyne incognita* after 7 days incubation

			Gall rating (0–4)[a] Soil heating[b]	
Amendment		Number of experiments	Nonheated	Heated
Bok choy	dried	2	0·00	0·08
	fresh	3	0·08	0·00
Broccoli	dried	3	0·86	0·00
	fresh	3	1·54	0·04
Cabbage	dried	2	0·81	0·06
	fresh	2	1·56	0·06
Cauliflower	dried	2	0·75	0·13
Black mustard	dried	3	2·21	0·17
Wild radish	dried	3	1·88	0·17
Non-amended control		8	3·55	3·63

[a] An arbitrary 0–4 rating scale was used where 0 = no visible galling; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100% of roots galled.

[b] The sublethal heating regime was a diurnal flux between 38°C maximum and 27°C minimum. The nontreated control temperature was 23°C (± 1°C).

deleterious effect on sclerotial germination, while black mustard, wild radish and cauliflower effects were not significantly different from the nontreated control. Addition of sublethal heating increased reductions in germination to 87–100%, with all amendments statistically of equal efficacy and all superior to the heated, non-amended control.

Soil amendment with each of the cruciferous residues except black mustard resulted in significantly reduced numbers of *P. ultimum* propagules after 2, 4 and 7 days.

Table 2 Effect of soil amendment with fresh or dried cruciferous plant residues, and/or sublethal heating, on germination of *Sclerotium rolfsii* sclerotia after 7 days incubation

			Germination (%)[a] Soil heating[b]	
Amendment		Number of experiments	Nonheated	Heated
Bok choy	dried	3	35·3 d[c]	13·2 b
	fresh	4	40·2 d	0·3 b
Broccoli	dried	5	52·9 cd	0·5 b
	fresh	4	63·9 cd	1·0 b
Cabbage	dried	3	61·9 bcd	0·4 b
	fresh	3	45·0 cd	0·1 b
Cauliflower	dried	2	98·0 ab	12·5 b
Black mustard	dried	3	75·2 abc	13·5 b
Wild radish	dried	2	78·1 abc	6·4 b
Non-amended control		8	99·9 a	94·2 a

Significance of main effects, interactions, and contrasts (*P*)

Source of variation	d.f.	Mean square
Amendment	(9)	4928·6***[d]
Amend vs. control	1	37129·3***
Dry vs. fresh	1	117·3 NS
Soil heat	1	28403·5***
Amend × heat	(9)	685·9 NS
Amend vs. con × heat	1	4387·2***
Dry vs. fresh × heat	1	14·9 NS
Error	54	364·5

[a] Germination percentage of sclerotia compared to nontreated control at time zero. All tests of statistical significance used arcsin of the square root of percentages. Time zero germination of sclerotia was 100%.

[b] The sublethal heating regime was a diurnal flux between 38°C maximum and 27°C minimum. The nontreated control temperature was 23°C (± 1°C).

[c] Common letters within columns denote values that are not different at $P = 0{\cdot}05$ by Fisher's Protected LSD test. Average observations per mean = 4.

[d] NS, not significant; ***$P < 0{\cdot}001$.

Table 3 Effect of cruciferous residues and/or sublethal soil heating on survival of *Pythium ultimum* after 7 days incubation

Amendment		Number of experiments	Survival (%)[a] Soil heating[b] Nonheated	Heated
Bok choy	dried	5	26·1 bc[c]	0·3 b
	fresh	4	6·0 d	0·0 b
Broccoli	dried	4	24·9 bc	2·8 b
	fresh	5	35·1 bc	1·2 b
Cabbage	dried	4	17·0 cd	0·2 b
	fresh	5	20·2 d	0·0 b
Cauliflower	dried	2	23·0 bc	0·0 b
Black mustard	dried	4	38·8 ab	3·5 b
Wild radish	dried	3	22·9 cd	0·0 b
Non-amended control		13	100·0 a	95·0 a

Significance of main effects, interactions, and contracts (*P*)

Source of variation	d.f.	Mean square
Amendment	(9)	4·2264***[d]
Amend vs. control	1	33·8059***
Dry vs. fresh	1	0·2563 NS
Soil heat	1	17·6008***
Amend × heat	(9)	0·6057***
Amend vs. con × heat	1	4·7040***
Dry vs. fresh × heat	1	0·0024 NS
Error	78	0·1542

[a] Percentage survival after 7 days incubation is the number of propagules per g of soil divided by the number in the non-amended, nonheated control on day 7. All tests of statistical significance used data transformed to $\log_{10}$ (% survival + 1).

[b] The sublethal heating regime was a diurnal flux between 38°C maximum and 27°C minimum. The nontreated control temperature was 23°C (± 1°C).

[c] Common letters within columns denote values that are not different at $P = 0{\cdot}05$ by Fisher's Protected LSD test. Average observations per mean = 5.

[d] NS, not significant; $^{***}P < 0{\cdot}001$.

Fresh bok choy and dried cabbage were the most effective amendments after 7 days of treatment (Table 3 and Fig. 1). Reductions after 2 days were from 26 to 61%, and after 7 days 52–91%. When the amendments were combined with the sublethal heating regime, the reductions increased to 54–100% after 2 days, and to 96–100% after 7 days with numbers of *P. ultimum* propagules reduced to undetectable levels in four of the 10 treatments tested (Table 3). As results from each of the three sampling times were similar, only the ANOVA table from the 7 days incubation is presented (Table 3). A representative summary of the dynamics of population decline of *P. ultimum* in soil amended with fresh bok choy or cabbage over the 7-day treatment period is shown (Fig. 1).

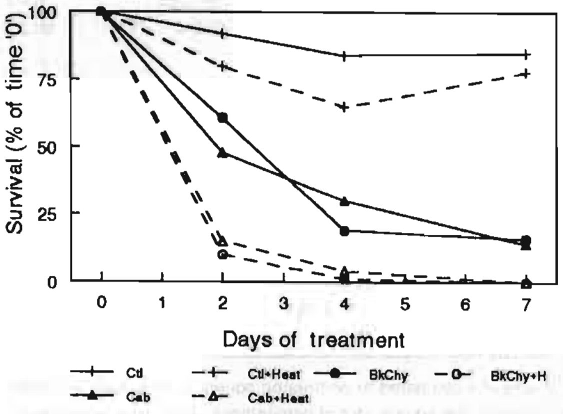

Figure 1 Dynamics of the effect of selected, fresh *Brassica* plant residues and/or sublethal soil heating on recovery of *Pythium ultimum* from treated soil. Ctl = non-amended control; BkChy = bok choy (*B. oleracea* var. *chinensis*); Cab = cabbage (*B. oleracea* var. *capitata*). Mean propagules of *P. ultimum* at time '0' = 28·7 per g dry soil.

The two non-cultivated cruciferous species tested, *B. nigra* (black mustard) and *R. sativus* (wild radish), which are common cool-season weeds in California, were generally the least effective as soil amendments for reducing activity of the test pathogens. All the cultivated Cruciferae tested, including bok choy, broccoli, cabbage and cauliflower, were effective in reducing activity of the test pathogens, with the exception that cauliflower had no effect on *S. rolfsii*.

None of the experiments showed differences in biotoxic effect of soil amendment with fresh versus dried cruciferous residues.

Discussion

Ramirez-Villapudua & Munnecke (1987, 1988) reported that dried cabbage was the best cruciferous amendment of the nine tested in combination with solarization for control of *Fusarium oxysporum* f.sp. *conglutinans*. Gamliel & Stapleton (1993b) demonstrated that cabbage residues combined with solarization provided excellent control of *P. ultimum* and *S. rolfsii* through production of biotoxic volatile compounds. The *in vitro* experiments described in this paper showed that amendment of infested soil with cruciferous residues (without soil heating) provided varying levels of activity against *M. incognita*, *P. ultimum* and *S. rolfsii*. Of the cruciferous amendments tested, bok choy and cauliflower had the best activity against *M. incognita* and *P. ultimum*; and fresh and dried bok choy and fresh cabbage were most effective against *S. rolfsii*. Our results also indicated that no clear advantage is gained through drying the crop residues prior to incorporation in soil. When combined with only a sublethal soil heating regimen, differences among cruciferous amendments were no longer found; all the species and varieties of cultivated and wild crucifers tested provided similar levels of improved pesticidal activity.

The biotoxic effects of volatile compounds from cruciferous residues, and particularly those of allyl isothiocyanate (Ellenby, 1945; Mayton *et al.*, 1996), have been amply demonstrated (Lewis & Papavizas, 1971, 1974; Gamliel & Stapleton, 1993b). However, the often low concentrations of pesticidal compounds produced during their decomposition without soil heating suggest that other modes of action, perhaps biological control of weakened propagules, also is likely to play a role in the process (Lifshitz *et al.*, 1983; Tjamos & Fravel, 1995; Davis *et al.*, 1996).

In developing feasible alternatives to chemical soil fumigants, it is essential that they provide reliable, predictable, and relatively rapid reductions of pathogen/pest inoculum. Solarization or addition of bioactive soil amendments alone may fulfil these requirements. However, in high-value, intensively farmed horticultural crops, it is unlikely that periodic rotations into bioactive plants will provide sufficiently effective or predictable soil disinfestation. Therefore, combination of soil amendments with solarization is a feasible option for development and implementation of effective soil fumigation.

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